

EFFECTIVENESS OF AN 80-20 FORMULATION OF CARBON
TETRACHLORIDE-METHALLYL CHLORIDE AS A FUMIGANT
FOR RICE WEEVIL, SITOPHILUS ORYZAE (L.), IN WHEAT

by

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INTRODUCTION

The search for effective stored grain fumigants has been in progress for many years. In part, the goal has been to find a fumigant which is effective against the internal infesting forms of insects as well as those on the outside of the kernel. With this point in mind, the tests herein reported were undertaken to determine the effectiveness of an 80-20 (by volume) formulation of carbon tetrachloride-methallyl chloride as a grain fumigant for adult and immature forms of the rice weevil, Sitophilus oryzae (L.).

REVIEW OF LITERATURE

Tests by Briejer (1938, 1939) showed methallyl chloride to be an effective fumigant against insects infesting stored products. He stated that granary weevils, Sitophilus (Calandra) granarius (L.), were not dead immediately after fumigation but that there was a strong after effect with all insects dying within a few days. Mention was also made of the ease with which it penetrated sacks of wheat to kill granary weevils. Richardson (1945) noted that the percentage of moribund cadelle, Tenebroides mauritanicus (L.), decreased progressively after fumigation with methallyl chloride in corn.

Richardson and Walkden (1945) reported tests using methallyl chloride, carbon tetrachloride, and various combinations of the two fumigants. They found methallyl chloride to be the more toxic compound to both rice weevils and confused flour beetles, Tribolium confusum Duv., in corn. Their data indicated a general decrease in toxicity of formulations as the percentage of methallyl chloride was decreased. Gallon jars containing five pounds of

corn were used as fumigation chambers with an exposure period of 24 hours at 30 degrees C.

Walkden and Schwitzgebel (1951) reported 100 percent mortality of insects in wheat fumigated under field conditions with an 80-20 formulation of carbon tetrachloride-methallyl chloride at a rate of 1.5 gallons per 1000 bushels. They observed no adverse effects on germination of wheat at this dosage.

Lindgren, et al. (1954) found methallyl chloride about equal in toxicity to rice weevils and saw-toothed grain beetles, Oryzaephilus surinamensis (L.), using two and six hour exposures. Granary weevils and lesser grain borers, Rhyzopertha dominica F., were also equal but required slightly higher dosages. In khapra beetle control studies, Lindgren, et al. (1955a) compared the toxicities of several fumigants to khapra beetles, Trogoderma granarium Everts, confused flour beetles, and granary weevils. Methallyl chloride was more toxic than carbon disulfide and ethylene dichloride but less toxic than methyl bromide, chloropicrin, and hydrogen cyanide.

Very little experimental data are available in the literature concerning the effects of fumigants on the immature stages of the internal infesting stored grain insects. Data that are available were obtained from studies with fumigants other than methallyl chloride or its combinations. Brierer (1938) reported that eggs, larvae, and pupae as well as adult granary weevils were killed by methallyl chloride but no specific data were mentioned. Lindgren, et al. (1955b) exposed khapra beetle eggs, larvae, and pupae to methallyl chloride for 2 hours at 70 degrees F. Data indicated pupae were more susceptible than larvae or eggs, eggs being the least susceptible. Adults were of the same order of susceptibility as pupae.

Adkisson (1957) reported the effects of carbon tetrachloride and ethylene

dichloride on all stages of the rice weevil. He found the order of susceptibility of the various stages to be different for each of the fumigants. Third instar larvae were least susceptible to ethylene dichloride and adults most susceptible. Carbon tetrachloride was most toxic to adults and least toxic to pupae, fourth instar larvae, and eggs.

Uses other than for fumigation of stored foodstuffs are also mentioned in the literature for methallyl chloride. Briejer (1941) reported control of the narcissus fly, Merodon equestris F., in plant bulbs with methallyl chloride. Brown (1951) indicated that methallyl chloride is a European soil fumigant and that it had been used to replace hydrogen cyanide in the fumigation of clothing for body louse control in Britain.

MATERIALS AND METHODS

Wheat

Source. Wheat for the fumigation tests was obtained from the Commodity Credit Corporation bin site at Clay Center, Kansas. Hard red winter wheat from the 1952 crop (variety unknown) was used on a loan basis from the bin site. Records indicated that it had not been fumigated or treated in any way for one year or more prior to its use.

Conditioning. Prior to testing, the wheat was made as uniform as possible by cleaning and tempering. A Seedmaster seed cleaner (PLATE I, Fig. 1) was used to remove chaff, cracked kernels, and other foreign material so that less than one percent dockage remained. After cleaning, the wheat was placed in a 55-gallon steel drum for tempering. Wheat for fumigation tests was used at three moisture levels: 11.0, 12.5, and 14.0 percent. To adjust the moisture to the desired level the following procedure was used.

EXPLANATION OF PLATE I

Fig. 1. Seedmaster seed cleaner.

Fig. 2. Electrically driven drum roller with drum in place.

PLATE I

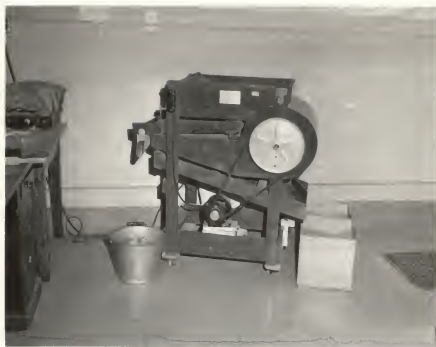


Fig. 1



Fig. 2

Moisture of the wheat to be tempered was determined by using a Steinlite Electronic Tester, Model G. It is advertised as correct to 0.25 percent (\pm). Periodically the moisture tester was checked against the standard air-oven method of moisture determination.¹ If necessary, a correction factor was applied to each moisture determination.

The amount of water required to raise the moisture of the wheat to that desired was calculated by using the original moisture and the desired. This amount of water was added to the wheat in the 55-gallon drum, the lid sealed with masking tape and the drum placed on an electrically operated drum roller (PLATE I, Fig. 2). Paddles welded to the inside of the drum aided the mixing of the wheat and water. Four to seven days were required to adjust the moisture to the desired level. Periodically during this time the drums were rolled for short periods of time to assure an even distribution of the moisture.

Insects

Rearing Procedure. Rice weevil adults and immature forms were reared in 13.0 percent moisture wheat in a rearing room at $80 \pm 2^{\circ}$ F. and 70 ± 5 percent relative humidity. Parent stock were obtained from cultures in existence at Kansas State College. One hundred parent adults were placed in each of several quart jars containing 100 grams of wheat (sterilized by freezing). The jars were covered with screened lids lined with filter paper. At the end of a 3-day oviposition period the adults were removed

¹ Official Methods of Analysis of the Association of Official Agricultural Chemists, Seventh Edition, 1950.

and the eggs laid during this period were allowed to develop. New cultures were started every three days to provide a constant supply of insects of the desired age.

Selection for Testing. Adults 14 to 21 days old were used for fumigation tests. Emergence of adult insects began about 30 days after the start of oviposition. Adults used were obtained from cultures approximately 49 days after parent stock had been placed in the wheat for oviposition.

Eggs, first instar larvae, third instar larvae, and pupae were the immature forms of the rice weevil used in the fumigation tests. The presence of each of these forms within a culture was determined by a schedule formulated by Adkisson (1956). By dissection and measurement of head capsule width he determined when the greatest percentage of a given form was present within the kernels of wheat. The schedule is as follows:

<u>Immature Form</u>	<u>Days after start of oviposition</u>
Eggs	3-4
First instar larvae	8-9
Second instar larvae	11-12
Third instar larvae	15-16
Fourth instar larvae	20-21
Pupae	25-26
Pre-emerged adults	27-30

O'Donnell (1956) reported similar data for the larvae when rice weevils were reared in wheat at $80 \pm 2^{\circ}$ F. and 70 ± 5 percent relative humidity.

On the basis of this information, cultures of wheat containing the desired immature form were selected for fumigation.

Fumigant

Chemical and Physical Properties. Methallyl chloride, $\text{CH}_2\text{C}(\text{CH}_3)\text{-CH}_2\text{Cl}$ (3-chloro-2-methyl-propene¹), is a colorless liquid with a rather strong petroleum-like odor. It boils at 72.2° C. and has a density (grams/milliliter) of 0.925 (one U. S. gallon weighs 7.7 lbs.). Solubility in alcohol and ether, etc. is infinite. Shepard (1951) stated its explosive range was 5.8 to 23.4 lbs. per 1000 cubic feet, a level seldom reached in practical fumigation. Synonyms: methylallyl chloride and isobutenyl chloride.

Carbon tetrachloride, CCl_4 (tetrachloromethane¹), is a colorless, non-inflammable liquid. It has a boiling point of 76.8° C. and a density of 1.595²⁰/₄ (one U. S. gallon weighs 13.3 lbs.). Solubility in ether, chloroform, and benzene is infinite. Solubility in water is 0.08 gms./100 ml. of water.

Formulation. An 80-20 (by volume) formulation of carbon tetrachloride-methallyl chloride was prepared by adjusting the temperature of each compound to 25° C. Quantities to give the 80 parts by volume of carbon tetrachloride and 20 parts by volume of methallyl chloride were measured by means of a 100 ml. graduate cylinder. A total of 500 ml. was formulated. The compounds were placed in a two liter amber glass bottle with a non-corrosive screw-type cap and placed in a refrigerator for storage (ca. 40° F.). The evaporation of either one of the compounds faster than the other to change the ratio (80-20) was considered negligible since the boiling points are similar, 72.2 and 76.8° C.

¹ Name approved by the International Union of Chemistry.

Pre-fumigation Preparation

Fumigation Chambers. One-gallon, narrow-mouthed glass jars were used as fumigation chambers. Self-sealing Mason lids were used to seal the chambers. A fine gauge steel wire, bent in a "U" shape, was soldered to the inner surface of the lid. It provided suspension for a half sheet of 9 cm. filter paper folded one time.

Insects, Cages, and Wheat. For fumigation tests with adult rice weevils, 50 insects, 14 to 21 days old, were placed in 60 mesh bronze wire-screen cages (3 inches long and 7/8 inches in dia.). Each cage contained 10 grams of wheat whose moisture was the same as that to be used in the fumigation chamber. Wax-coated corks were used to stopper the cages.

Cultures of immature rice weevil to be fumigated were selected as previously described. A quantity of wheat, containing the desired immature form, sufficient to provide two 10-gram samples per fumigation chamber was selected. It was thoroughly mixed by making three passes through a Boerner grain divider. Ten gram samples of the wheat were then placed in the wire cages and stoppered with wax-coated corks.

Wheat, 1980 grams, at the desired moisture was weighed out and placed in each fumigation chamber in the following manner. First, 990 grams of wheat were placed in the chamber. Then two cages containing test insects were placed in the center of the jar with the long axis parallel to the surface of the wheat. An additional 990 grams of wheat were then placed in the jar and the surface leveled. The two 990 gram portions plus the ten grams of wheat in each cage totaled 2000 grams per fumigation chamber. Two cages of 50 adult insects each gave a total of 100 insects per dosage per test. The number of immature insects per dosage per test varied.

Conditioning Period. After the self-sealing lids with filter paper folds were put in place, the chambers were stored for approximately 24 hours at $80 \pm 2^\circ$ F. Pre-conditioning in this manner allowed the atmosphere (temperature and humidity) within the chambers to stabilize. It also allowed the insects to become accustomed to the environment desired during the exposure period.

Fumigant Application and Exposure

Application Methods. A method whereby small increments of fumigant could be accurately measured and delivered to the fumigation chambers rapidly was required. Methods which were tried included use of (1) a 1 cc. tuberculin syringe graduated in units of 0.01 ml., (2) micro pipettes of various sizes, and (3) fumigant dilution with acetone. None of these methods met the requirements. The fourth method, a micro syringe, met the requirements.

Micro Syringe. A micro syringe (PLATE II, Fig. 1) was used to measure the fumigant and place it in the chambers. The syringe consists of a precision bore tube of "Pyrex" brand glass into which a Teflon¹ plunger has been accurately fitted. A stainless steel shaft extends from the plunger to the micrometer control, graduated in 0.0001 ml. units. The delivery end of the syringe is a modified 27 gauge dental-type hypodermic needle. Accurate alignment of the components of the syringe is assured by a slotted stainless steel tube which fits over the glass syringe barrel. The slotted tube screws into an anodized aluminum micrometer housing thus facilitating rapid disassembly for cleaning.

¹ Trademark for DuPont Tetrafluoroethylene.

EXPLANATION OF PLATE II

Fig. 1. Micro syringe used for fumigant application.

Fig. 2. Application of fumigant to fumigation chamber.

PLATE II



Fig. 1

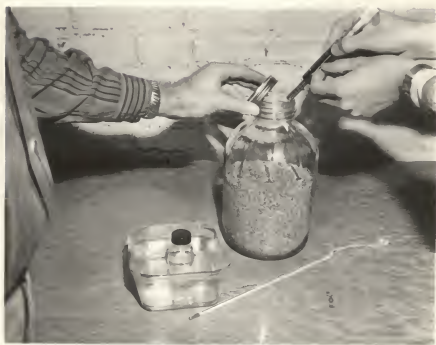


Fig. 2

Fumigant Application. At the time of fumigation a small quantity of the fumigant formulation was removed from the amber glass storage bottle and placed in a two ounce bottle. Temperature was adjusted to 25° C. by placing the two ounce bottle of the formulation in a water bath. Room temperature was also adjusted to approximately 25° C. to minimize temperature change in the fumigant during application.

Chambers to be fumigated were removed from the conditioning storage area. Fumigant was applied to each chamber in the following manner.

The syringe needle was inserted into the fumigant formulation and the syringe filled by drawing back the plunger. It was filled and emptied several times prior to fumigating any chamber. The fumigant was measured by filling the syringe and adjusting the micrometer to the desired dosage. Excess fumigant was expelled during this operation. The lid of the chamber was lifted just enough to allow the syringe to be inserted (PLATE II, Fig. 2). By a downward movement of the syringe plunger, the fumigant was placed on the filter paper fold suspended from the lid. The syringe was rapidly withdrawn and the lid replaced, sealing the chamber.

Exposure. After the fumigant had been applied, the chambers were returned to the constant temperature storage area (80 \pm 2° F.) and exposed for 48 hours. All of the fumigation tests herein reported were exposed for 48 hours. At the end of the exposure period, cages containing test insects were removed from the chambers and aerated for one hour.

Mortality Determination

Adults. After the aeration, mortality counts were made to determine the number of live and dead insects. Criterion for death was total immobility, i.e., any movement whatsoever classed the insect as live. The numbers

so determined were recorded as 0-day mortality. The insects were placed in one ounce tin salve boxes containing 10 grams of clean, sterilized wheat at 13.0 percent moisture. Each salve box was covered by a screened lid held in place by an identification tape.

All salve boxes for a given fumigation test were buried in 15 pounds of wheat, 13.0 to 14.0 percent moisture content, contained in a 30-pound capacity frozen food can. This was done to provide an environment as near normal as possible during the recovery period. The lid of the can was sealed with masking tape to exclude other insects and to maintain the moisture of the wheat. The cans were stored at $80 \pm 2^{\circ}$ F. After five days, the insects were removed, the mortality determined, and the insects replaced as previously described. This constituted the 5-day count. At the end of 10 days the mortality was again determined and the insects discarded.

Immature Forms. After aeration, the 10-gram samples of wheat containing the immature forms were placed in one ounce tin salve boxes. Each was covered with a screened lid held in place by an identification tape. All samples were buried in wheat following the same procedure as that for the adult rice weevils. They were also stored at $80 \pm 2^{\circ}$ F.

At approximately 32 days from the start of oviposition for a given immature form, the samples were removed from storage and the number of emerged adults recorded. The samples were replaced in storage. Six counts at intervals of five days were made. The total emergence at each level of treatment was determined by summing the number of emerged insects from the six counts. In determining the percentage mortality of the immature forms, the total number of adult rice weevils emerging from each treated sample (E) was subtracted from the number emerging from its respective control sample. The predicted number of dead insects (PD), estimated in this way, was then

divided by the total number of insects emerging from the control samples to determine the percentage mortality.

RESULTS AND DISCUSSION

Adults

Three separate fumigation tests using adult rice weevils were run at each moisture level. Approximately 300 insects were used for each treatment level and control within the effective range of the fumigant. Tables 1, 2 and 3 present mortality data collected from tests conducted in wheat of 11.0, 12.5 and 14.0 percent moisture respectively. Percentage mortality for each test and the average total percentage mortality are given for counts made 0, 5 and 10 days from the end of the exposure period. Mortalities in treated samples were not corrected for natural mortality. Four percent control mortality was recorded in two tests and 5.2 percent in another, however, in no instance did the average total percentage mortality exceed 2.0 percent.

There is, in general, only a slight difference in the average total percentage mortality for the 5- and 10-day counts. Between the 0- and 5-day counts, however, there is an appreciable difference especially near the center of the effective range of the fumigant (near LC_{50}). This indicates that within five days after fumigation, under the conditions stated, most of the insects that will die as a result of the fumigation will have been killed. There is no evidence of an initial "knock-down" with subsequent recovery but rather the data suggest that some insects that eventually die are still active on the initial count. These results show the same effect with rice weevils that was found by Briejer (1938) with granary weevils and

Table 1. Percentage mortality of adult rice weevils when fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride for 48 hours at 80 degrees F. in 11.0 percent moisture wheat.

µl./2000 gms.	O-day				5-day				10-day			
	percentage mortality				percentage mortality				percentage mortality			
	Replicates				Replicates				Replicates			
	(1)	(2)	(3)	Av.	(1)	(2)	(3)	Av.	(1)	(2)	(3)	Av.
5	1.0	0.0	*	**	0.0	0.0	*	**	1.0	0.0	*	**
10	1.9	1.0	*	**	2.9	1.0	*	**	2.9	2.0	*	**
15	1.0	1.0	*	**	3.0	1.0	*	**	5.0	1.0	*	**
20	2.0	1.0	*	**	2.0	2.0	*	**	2.0	2.0	*	**
25	1.0	0.0	14.6	5.1	1.0	0.0	24.0	8.2	2.1	1.0	25.0	9.2
30	2.0	3.7	24.0	9.8	9.2	15.0	38.0	20.6	12.2	12.1	38.0	20.7***
35	2.1	5.8	20.0	9.4	10.5	16.5	29.7	19.1	16.0	10.7	30.7	19.1***
40	8.0	5.0	31.0	14.7	40.4	27.0	49.0	38.8	42.4	31.0	43.0	42.1***
45	19.2	13.0	41.4	24.5	45.4	43.0	61.6	50.0	49.5	45.4	61.2	52.0***
50	30.9	25.0	62.0	39.4	63.5	65.6	86.9	71.6	71.1	69.7	89.9	76.9***
55	59.4	42.0	82.8	61.3	71.3	77.0	91.0	79.7	75.2	80.0	91.0	82.0***
60	67.7	65.6	88.9	74.3	89.9	86.9	95.9	90.9	89.9	87.0	96.0	91.2***
65	89.7	84.0	98.0	90.6	95.9	95.0	98.0	96.3	96.9	95.0	98.0	96.6
70	95.2	99.0	99.0	97.7	99.0	100.0	100.0	99.7	100.0	100.0	100.0	100.0
75	97.0	99.0	100.0	98.7	100.0	100.0	99.0	99.7	100.0	100.0	100.0	100.0
80	100.0	99.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
85	100.0	100.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
90	100.0	100.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
95	100.0	100.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
Control	0.0	0.0	1.0	0.3	0.0	1.0	5.2	2.0	1.0	1.0	2.1	1.4

* Dosage omitted in replicate (3).

** Average percentage mortality not calculated.

*** Data used in IEM-650 analysis.

Table 2. Percentage mortality of adult rice weevils when fumigated with an 80-20 formulation of carbon tetrachloride-methylal chloride for 48 hours at 80 degrees F. in 12.5 percent moisture wheat.

μl./2000 gms.	0-day			5-day			10-day		
	percentage mortality			percentage mortality			percentage mortality		
	Replicates	(1)	(2)	(3)	Av.	Replicates	(1)	(2)	(3)
					Av.				Av.
10	0.0	0.0	0.0	*	**	1.0	0.0	0.0	**
15	0.0	0.0	0.0	*	**	0.0	2.0	1.0	**
20	1.0	0.0	0.0	*	**	2.0	1.0	1.0	*
25	1.0	1.0	0.0	*	**	1.0	5.0	1.0	*
30	2.0	0.0	0.0	*	**	3.9	0.0	0.0	*
35	1.0	0.0	0.0	*	**	2.0	0.0	0.0	**
40	0.0	1.0	0.0	*	**	1.0	7.1	0.0	**
45	1.0	2.0	3.0	*	2.0	2.0	3.0	1.0	**
50	1.0	6.9	10.9	*	6.4	5.0	5.9	4.9	6.0
55	4.8	9.8	15.8	*	10.1	9.6	10.0	3.0	6.4***
60	7.1	20.8	28.4	*	18.9	21.4	29.7	9.5	13.4***
65	31.0	29.2	36.4	*	32.2	61.6	54.2	21.6	29.4***
70	42.3	51.0	69.6	*	54.5	67.7	64.0	63.3	56.2***
75	55.4	54.6	93.1	*	68.0	84.2	74.2	66.7	70.0***
80	86.1	84.3	94.0	*	88.4	93.1	95.1	84.2	86.3***
85	99.0	94.9	99.0	*	97.6	100.0	99.0	95.0	96.0***
90	96.9	99.0	*	*	**	98.0	99.0	100.0	99.0
95	100.0	99.0	*	*	**	100.0	100.0	98.0	**
100	100.0	99.0	*	*	**	100.0	100.0	100.0	**
Control	0.0	0.0	1.0	0.0	0.3	0.0	0.0	0.0	1.3

* Dosage omitted in replicate (3).

** Average percentage mortality not calculated.

*** Data used in IBM-650 analysis

Table 3. Percentage mortality of adult rice weevils when fumigated with an 80-20 formulation of carbon tetrachloride-methylal chloride for 48 hours at 80 degrees F. in 14.0 percent moisture wheat.

µl./2000 gms.	0-day			5-day			10-day			AV.		
	percentage mortality			percentage mortality			percentage mortality					
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)			
40	3.9	6.2	0.0	3.3	3.9	7.2	0.0	3.7	3.9	7.2	3.0	4.7
45	1.0	6.7	3.0	3.6	4.0	7.8	7.1	6.3	3.0	9.7	12.4	8.4
50	14.0	3.0	2.9	6.6	15.0	3.1	5.8	8.0	17.0	4.1	7.8	9.6
55	8.9	7.1	9.1	8.4	9.9	9.2	8.0	9.0	11.9	10.2	9.0	10.4***
60	10.9	25.2	12.9	16.6	13.9	29.6	9.0	17.4	14.8	30.9	10.2	18.6***
65	12.0	20.4	17.5	16.6	15.0	26.8	21.0	20.9	16.0	26.8	19.0	20.6***
70	21.4	15.4	21.0	19.4	27.6	22.8	omit	25.2	27.8	23.9	omit	25.8***
75	30.3	31.6	37.2	31.3	44.4	42.8	39.2	42.2	46.9	44.9	44.9	45.6***
80	53.9	46.6	43.7	48.0	58.8	53.9	54.4	55.7	59.8	55.4	56.3	57.2***
85	59.0	60.6	69.0	63.6	72.4	59.6	69.8	67.4	72.4	58.5	70.4	67.1***
90	78.6	73.7	55.0	69.0	90.8	78.8	58.9	76.4	92.8	78.8	65.0	78.9***
95	81.6	87.1	86.1	85.0	90.8	97.9	93.0	93.8	91.8	97.7	94.0	94.6***
100	88.1	89.0	89.3	88.8	96.0	96.0	93.8	95.3	95.0	97.0	94.7	95.6
105	98.0	94.9	95.3	96.0	100.0	99.0	99.0	99.3	100.0	97.7	99.0	99.0
110	99.0	97.1	99.0	98.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
115	100.0	100.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
120	100.0	100.0	*	**	99.0	100.0	*	**	100.0	100.0	*	**
125	100.0	100.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
130	100.0	100.0	*	**	100.0	100.0	*	**	100.0	100.0	*	**
Control	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.3	2.0	1.0	0.0	1.0

* Dosage omitted in replicate (3).

** Average percentage mortality not calculated.

*** Data used in IRI-650 analysis.

Richardson (1945) with cadellies using methallyl chloride. In contrast, some other fumigants do cause an anesthetic effect with a subsequent recovery and other fumigants require an appreciably longer period before the final mortality can be determined.

The dosages ($\mu\text{l}/2000$ grams of wheat) required for 100 percent mortality at each moisture level are as follows:

<u>Percent Moisture</u>	<u>0-day</u>	<u>5-day</u>	<u>10-day</u>
11.0	85	75	70
12.5	>100	100	95
14.0	115	110	110

These values are based on tests (1) and (2) in Tables 1, 2 and 3 since test (3) did not cover the entire range of dosages and did not, in all cases, reach the 100 percent mortality limit.

An IBM 650 Magnetic Drum Data Processing Machine (hereafter referred to as the IBM 650) was used to statistically analyze the dosage mortality data. Sokal (1958) prepared a program for this computer whereby it would process dosage-mortality data by the method of maximum likelihood. Table 4 is a compilation of LC_{50} values, b values (slopes), the upper and lower fiducial limits of LC_{50} and b at 0.05 probability, and estimated LC_{90} values for adult and immature forms of the rice weevil. The LC_{90} values are estimated from dosage mortality curves plotted in PLATES III, IV and V. In addition to the information just mentioned, the IBM 650 also calculated the mortality probits for each dosage used in the analysis.

PLATE III is a graphic presentation of the 10-day dosage-mortality data as analyzed by the IBM 650. Mortality curves are plotted on logarithmic-probit graph paper so the data may be observed in a straight line relationship. Relative positions of the three curves (11.0, 12.5 and 14.0

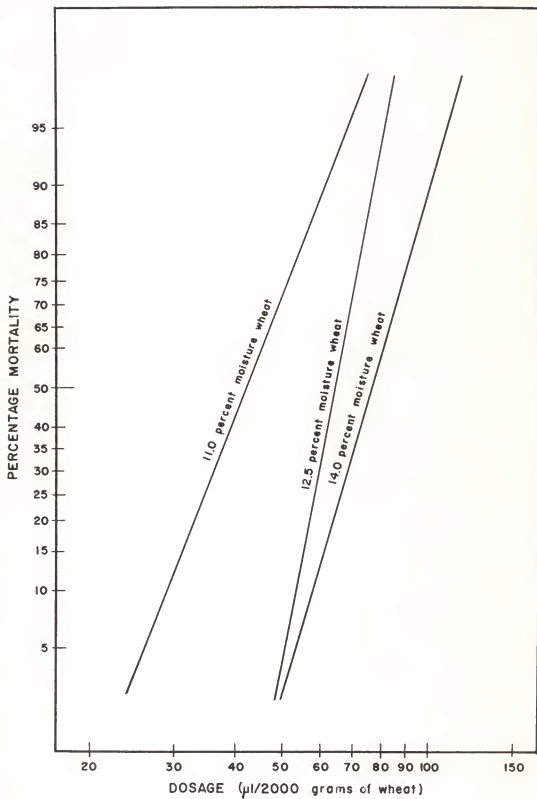
Table 4. Dosages of an 80-20 (by volume) formulation of carbon tetrachloride-methyllyl chloride required to kill 50 and 90 percent of rice weevil, *Sitophilus oryzae* (L.), adult and immature forms when exposed for 48 hours at 80 \pm 2 degrees F. in 2000 grams of wheat at two moisture levels.

Moisture of wheat	Stage	Slopes			µl/2000 gms of wheat			
		bl	b	bu	LC ₅₀	LC ₅₀	LC ₅₀	LC ₉₀ (est.)
11.0%	Adult	5.42	7.76	10.10	39.03	41.94	44.73	61.4
	Egg	8.29	10.89	13.49	75.97	79.62	83.13	104.0
	1st Instar	5.65	9.35	13.05	74.39	81.06	87.52	111.0
	3rd Instar	11.39	15.51	19.63	100.47	104.35	108.27	126.0
	Pupa	4.60	5.11	5.61	58.67	61.21	63.51	109.0
14.0%	Adult	14.82	15.96	17.10	63.43	64.03	64.63	77.0
	Egg	8.28	9.24	10.20	91.55	93.43	95.27	129.0
	1st Instar	10.75	12.69	14.62	111.34	114.05	116.68	144.0
	3rd Instar	7.37	11.12	14.90	130.73	137.12	145.22	180.0
	Pupa	6.60	7.24	7.87	98.76	100.94	102.98	133.0
	Adult	8.40	10.76	13.13	72.86	75.58	78.43	101.0

EXPLANATION OF PLATE III

Dosage-mortality curves for rice weevil fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride in wheat at 11.0, 12.5 and 14.0 percent moisture.

PLATE III



percent moisture mortalities) shows a direct relationship between moisture of the wheat and quantity of the fumigant to kill adult rice weevils. As the moisture of the wheat is increased, the quantity of fumigant to kill a given percentage of insects is increased. In general, this relationship is true for most fumigants.

Carbon tetrachloride-carbon disulfide (80-20 by volume) has often been referred to as the "standard" of grain fumigants. By comparing carbon tetrachloride-methallyl chloride with this "standard", an estimation of its relative effectiveness can be obtained. This comparison is made in Table 5. The LC_{50} values for carbon tetrachloride-methallyl chloride were calculated by the IBM 650 and LC_{90} values were estimated from the plotted curves in PLATE III. The corresponding values for carbon tetrachloride-carbon disulfide were calculated by O'Donnell (1958) from data obtained under essentially the same conditions as those described in this thesis. LC values for 11.0 percent moisture wheat show no differences between the two formulations. In 12.5 percent moisture wheat, carbon tetrachloride-methallyl chloride required 6.7 μ l. less fumigant to kill 50 percent of the insects and 27.3 μ l. less to kill 90 percent of the insects. Both formulations require about the same amount to kill 50 percent of the insects in 14.0 percent moisture wheat but to kill 90 percent, carbon tetrachloride-methallyl chloride required 32.2 μ l. less.

Immature Forms

Three separate fumigation tests were also made for each immature stage at each dosage and at two moisture levels, 12.5 and 14.0 percent. Total weight of infested wheat per dosage per moisture was 60 grams. Total numbers of each stage emerging from the control samples ranged from 181 to 321 and are

Table 5. Comparison of LC_{50} and LC_{90} values for 80-20 formulations of carbon tetrachloride-methallyl chloride and carbon tetrachloride-carbon disulfide when used as a fumigant for rice weevil in 11.0, 12.5 and 14.0 percent moisture wheat (48 hour exposure at 80 \pm 2 degrees F.).

80-20 Fumigant Formulation	LC	µl/2000 grams of wheat		
		11.0%	12.5%	14.0%
Carbon tetrachloride- methallyl chloride	LC_{50}	41.9	64.0	75.6
	LC_{90}	61.4	77.0	101.0
Carbon tetrachloride- carbon disulfide*	LC_{50}	40.1	70.7	75.0
	LC_{90}	60.4	104.3	133.2

* Note: These values from tests by A. E. O'Donnell (1958).

indicated in Tables 6 and 8.

Laboratory fumigation tests of internal infesting immature insects have not been extensive, primarily because of the difficulty in ascertaining the results. The method used in these tests, previously described, is considered to be valid.

Tables 6 and 8 show the total number of emerged insects (E) and the predicted number of dead insects (PD) from each treated and control sample for tests in 12.5 and 14.0 percent moisture wheat respectively. The sums of emerged and predicted numbers of dead insects for the three tests at each treatment level for a given immature stage are also included. These sums were used in obtaining the LC_{50} and b values with the IBM 650. When the number of insects emerging from a treated sample was greater than that in the respective control sample, the number of dead individuals could not be predicted and is indicated by a dash (-) in Tables 6 and 8.

Tables 7 and 9 are summaries of the percentage mortalities obtained when the various immature stages were fumigated in 12.5 and 14.0 percent moisture wheat respectively. The mortality for each dosage in each test

Table 6. Number of emerged (E) and predicted number of dead (PD) insects for each treated and control sample fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride for 48 hours at 80 \pm 2° F. in 12.5 percent moisture content wheat.

Stage	Dosage μl/2000 gms	Replicates								Total	
		(1)		(2)		(3)					
		E.	P.D.	E.	P.D.	E.	P.D.	E.	P.D.	E.	P.D.
Eggs	Control	96	0	109	0	79	0	284	0		
	40	96	0	113	-	omitted		omitted			
	50	116	-	110	-	77	2	303	-		
	60	78	18	102	7	66	13	246	38*		
	70	51	45	90	19	79	0	220	64*		
	80	26	70	81	28	50	29	157	127*		
	90	8	88	32	77	32	47	72	212*		
	100	3	93	20	89	9	70	32	252*		
	110	0	96	11	98	8	71	19	265*		
1st Instar Larvae	Control	97	0	68	0	118	0	283	0		
	40	86	11	77	-	129	-	292	-		
	50	107	-	73	-	121	-	301	-		
	60	72	25	72	-	105	13	249	34*		
	70	59	39	47	21	76	42	182	101*		
	80	46	51	54	14	76	42	176	107*		
	90	22	75	29	39	64	54	115	168*		
	100	5	92	15	53	36	82	56	227*		
	110	0	97	0	68	13	105	13	270*		
3rd Instar Larvae	Control	145	0	95	0	77	0	317	0		
	60	113	32	101	-	71	6	285	32		
	70	124	21	97	-	70	7	291	26		
	80	120	25	97	-	77	0	294	23*		
	90	110	35	107	-	55	22	272	45*		
	100	83	62	84	11	40	37	207	110*		
	110	38	107	57	38	32	45	127	190*		
	120	19	126	24	71	13	64	56	261*		
	130	0	145	7	88	2	75	9	308*		
Pupae	Control	86	0	108	0	82	0	276	0		
	50	omitted		69	39	60	22	129**	61*		
	60	omitted		51	57	32	50	83**	107*		
	70	48	38	47	61	23	61	116	160*		
	80	32	54	26	82	26	56	84	192*		
	90	29	57	12	96	12	70	53	223*		
	100	16	70	12	96	15	68	43	233*		
	110	11	75	8	100	7	75	26	250*		
	120	6	80	4	104	5	77	15	261*		
	130	5	81	6	102	0	82	11	265*		
	140	6	80	2	106	6	76	14	262*		

* Data used in IBM 650 analysis.

** 190 used as control emergence.

Table 7. Percentage mortality of four immature stages of the rice weevil, *Sitophilus oryzae* L., when fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride for 48 hours at 80 degrees F. in 12.5 percent moisture wheat.

Immature Stage	:	(pl/2000 gms)	:	Replicates			:	Average Total		
	:		:	(1)	:	(2)	:	(3)	:	
Eggs		40		-		-		-		-
		50		-		-		2.5		-
		60		18.8		6.4		16.4		13.4
		70		46.9		17.4		0.0		22.5
		80		72.9		25.7		36.7		44.7
		90		91.7		70.6		59.5		74.6
		100		96.9		81.6		88.6		80.7
		110		100.0		89.9		89.9		93.3
1st Instar Larvae		40		11.3		-		-		-
		50		-		-		-		-
		60		25.8		-		11.0		12.0
		70		40.2		30.9		35.6		36.0
		80		52.6		20.6		35.6		37.8
		90		77.3		57.4		45.8		59.4
		100		94.8		77.9		69.5		80.2
		110		100.0		100.0		89.0		95.4
3rd Instar Larvae		60		22.1		-		7.8		10.1
		70		14.5		-		9.1		8.2
		80		17.2		-		0.0		7.2
		90		24.1		-		28.6		14.2
		100		42.8		11.6		48.0		34.7
		110		73.8		40.0		58.4		59.9
		120		86.9		74.7		83.1		82.3
		130		100.0		92.6		97.4		97.2
Pupae		50		-		36.1		26.8		32.1
		60		-		52.8		61.0		56.3
		70		44.2		56.5		74.4		58.0
		80		62.8		75.9		68.3		69.6
		90		66.3		88.9		85.4		80.8
		100		81.4		88.9		82.9		84.4
		110		87.2		92.6		91.5		90.6
		120		93.0		97.3		93.9		94.6
		130		94.2		94.4		100.0		96.0
		140		93.0		98.1		92.7		94.9

Table 8. Number of emerged (E) and predicted number of dead (PD) insects for each treated and control sample fumigated with an 80-20 formulation of carbon tetrachloride-methylal chloride for 48 hours at 80 \pm 2° F. in 14.0 percent moisture content wheat.

Stage	Dosage µl/2000 gms	Replicates						Total	
		(1)		(2)		(3)		E.	P.D.
		E.	P.D.	E.	P.D.	E.	P.D.		
Eggs	Control	59	0	48	0	74	0	181	0
	60	48	11	48	0	86	-	182	-
	70	38	21	32	16	79	-	149	32*
	80	40	19	39	9	65	9	144	37*
	90	31	28	26	22	50	24	107	74*
	100	15	44	19	29	36	38	70	111*
	110	16	43	8	40	19	55	43	138*
	120	6	53	6	42	17	57	29	152*
	130	2	57	2	46	11	63	15	166*
	140	3	56	1	47	8	66	12	169
	150	1	58	1	47	1	73	3	178
	160	0	59	0	48	2	72	2	179
1st Instar Larvae	Control	131	0	70	0	108	0	309	0
	80	120	11	73	3	110	-	303	6
	90	127	4	54	16	91	17	272	37*
	100	105	26	60	10	73	35	238	71*
	110	74	57	48	22	55	53	177	132*
	120	67	64	38	32	37	71	142	167*
	130	26	105	32	38	12	96	70	239*
	140	21	110	9	61	11	97	41	268*
	150	3	128	6	64	3	105	12	297*
	160	0	131	3	67	1	107	4	305
3rd Instar Larvae	Control	142	0	103	0	76	0	321	0
	80	128	14	87	16	87	-	302	19
	90	119	23	93	10	59	17	271	50
	100	108	34	79	24	96	-	283	38*
	110	103	39	87	16	85	-	275	46*
	120	93	49	86	17	77	-	256	65*
	130	85	57	68	35	64	12	217	104*
	140	62	80	57	46	45	31	164	157*
	150	11	131	40	63	25	51	76	245*
	160	12	130	39	64	17	59	68	253*

Table 8. (concl.)

Stage	pl/2000 gms	Replicates							
		(1)		(2)		(3)		Total	
		E.	P.D.	E.	P.D.	E.	P.D.	E.	P.D.
Pupae	Control	107	0	82	0	63	0	252	0
	80	82	25	66	13	42	21	190	62*
	90	58	49	64	15	38	24	160	92*
	100	61	46	37	42	27	36	125	127*
	110	31	76	48	31	27	36	106	146*
	120	29	78	32	47	17	46	78	174*
	130	19	88	16	63	20	43	55	197*
	140	17	90	23	56	9	54	49	203*
	150	5	102	12	67	4	59	21	231*
	160	2	105	2	77	8	55	12	240*
	170	1	106	4	75	8	55	13	239
	180	1	106	1	78	5	58	7	245

* Data used in IBM 650 analysis.

Table 9. Percentage mortality of four immature stages of the rice weevil, *Sitophilus oryzae* L. when fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride for 48 hours at 80 degrees F. in 14.0 percent moisture wheat.

Immature Stage	(µl/2000 gms)	Replicates			Average Total
		(1)	(2)	(3)	
Eggs	60	18.6	30.0	-	-
	70	35.6	33.3	-	17.7
	80	32.2	18.8	12.2	20.4
	90	47.4	45.8	32.4	40.9
	100	74.6	60.4	51.4	61.3
	110	72.9	83.3	74.3	76.2
	120	89.8	87.5	77.0	84.0
	130	96.6	95.8	85.1	91.7
	140	94.9	97.9	89.2	93.4
	150	98.3	97.9	98.6	98.3
	160	100.0	100.0	97.3	98.9
1st Instar Larvae	80	8.4	4.1	-	1.9
	90	3.0	22.8	15.7	12.0
	100	19.8	14.3	32.4	23.0
	110	43.5	31.4	49.1	42.7
	120	48.8	45.7	65.7	54.0
	130	80.2	54.3	88.9	77.3
	140	84.0	87.1	89.8	86.7
	150	97.7	91.4	97.2	96.1
	160	100.0	95.7	99.1	98.7
3rd Instar Larvae	80	9.8	15.5	-	5.9
	90	16.2	9.7	22.4	15.6
	100	23.9	23.3	-	11.8
	110	27.5	15.5	-	14.3
	120	34.5	16.5	-	20.2
	130	40.1	34.0	15.3	32.4
	140	56.3	44.7	40.8	48.9
	150	92.2	61.2	67.1	76.3
	160	91.5	62.1	77.6	78.8
Pupae	80	23.4	15.8	33.3	24.6
	90	45.8	18.3	39.7	36.5
	100	43.0	51.2	57.1	50.4
	110	71.0	37.8	57.1	57.9
	120	72.9	57.3	73.0	69.0
	130	82.2	76.8	68.2	78.2
	140	84.1	68.3	85.7	80.6
	150	95.3	81.7	93.6	91.7
	160	98.1	93.9	87.3	95.2
	170	99.1	91.5	87.3	94.8
	180	99.1	95.1	92.1	97.2

is given as well as the average total percentage mortality for each dosage. A dash (-) is also used in these tables when the number of insects emerging from the treated samples exceeded those emerging from the respective control.

Information obtained by processing the data with the IBM 650 is tabulated with that of the adults in Table 4. The LC_{90} values are estimated from the dosage mortality curves in PLATES IV and V. Upper and lower fiducial limits of the LC_{50} and b values are indicated by the subscripts u and l respectively. These limits denote the 95 percent fiducial limits, i.e., if the tests were repeatedly tried, the results would be expected to fall within the limits 19 out of 20 times or 95 percent of the time.

When the LC_{50} values and their upper and lower fiducial limits of the various stages are compared, the order of susceptibility is as follows:

12.5 percent moisture wheat

least susceptible - third instar larvae
 - first instar larvae and eggs
 most susceptible - pupae (and adults)

14.0 percent moisture wheat

least susceptible - third instar larvae
 - first instar larvae
 - pupae
 - eggs
 most susceptible - (adults)

At both moistures, the third instar larvae are the least susceptible or hardest to kill. Adkisson (1956) also observed the third instar larvae as hardest to kill when fumigated with ethylene dichloride. He stated that third instar larvae were generally found deepest within the kernel and speculated that the degree of resistance might be due to their relative position within the kernel. Sun (1947) found that larvae of confused flour beetles, when fumigated with carbon disulfide, were least susceptible about midway

through the larval stage. These larvae are not internal infesting forms and do not have the protective coating of the wheat kernel that the rice weevil has in the immature stages, yet it appears least susceptible at about the same point in larval development. There are innumerable factors which might be responsible for one stage of an insect being less susceptible than another. Sun (1947) and other authors believe the susceptibility of insects depends mainly on the rate of respiration. Lindgren (1935), on the other hand, found that this relationship did not necessarily apply in all cases and thought the fumigant was the determining factor. Sun further stated that physical factors not only affect the respiration but also the absorption, adsorption, diffusion, permeation, volatility, chemical reaction, water loss, etc. and that these factors more or less affect the toxicity of fumigants to insects.

Eggs and first instar larvae are classed as equally susceptible when fumigated in 12.5 percent moisture wheat since the LC_{50} upper and lower limits of the egg and first instar larvae, respectively, overlap.

Order of susceptibility varies with respect to moisture of the wheat for the eggs and pupae. The reason for this change is not known. There is also an apparent separation in the susceptibilities of the various immature forms in the 14.0 percent moisture wheat to form five separate groups, i.e., there is no overlap of susceptibility at this moisture. At the 12.5 percent moisture level, however, the eggs and first instar larvae overlap as do the pupae and adults.

Slopes (b values) for each of the mortality curves are included in Table 4. These values are indicators of the relative inclination of the curves. The greater the slope, the more vertical the curve. As the slope

increases, the amount of fumigant required to increase the mortality from 50 to 90 percent decreases. Slope measures the vertical increase for one unit of horizontal increase (mortality increase per unit of dosage). The slopes, in general, do not conform to a definite pattern for these tests. Pupae, however, in both moistures of wheat, as shown graphically in PLATES IV and V, have the smallest values.

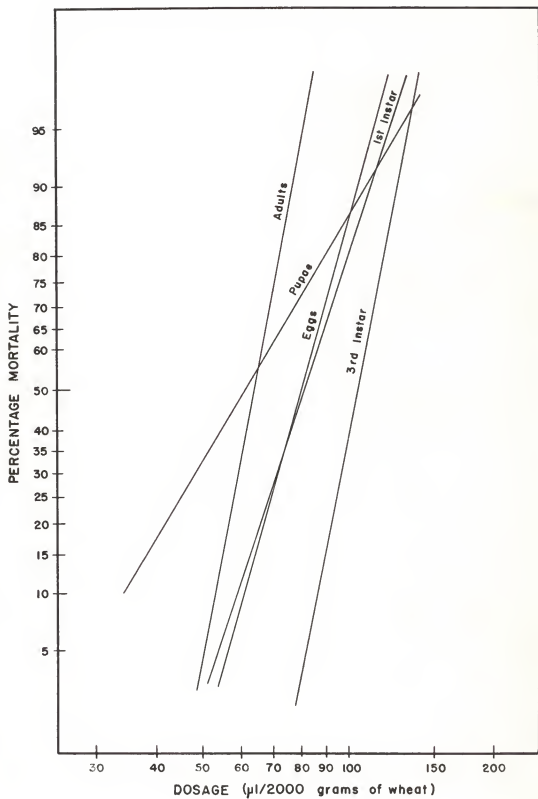
Upper and lower fiducial limits of b and LC_{50} show the third instar larvae to have the greatest overall variability. It is probably, in part, due to the overlap of larval instars found in the fumigated samples containing the third instar larvae. By dissecting kernels of wheat 15 days after the start of oviposition, O'Donnell (1956) found that 82.5 percent of the larvae were third instar, 5.5 percent were second instar, and 10.5 percent were fourth instar. Kernels dissected 8 days after the start of oviposition showed 98.2 percent of the larvae present to be first instar larvae. They ranked second in extent of overall variability. Eggs and pupae did not have as much variability as did first and third instar larvae. Eggs take four to five days to hatch so that fumigating wheat containing eggs, four days after the start of oviposition, 100 percent eggs could be assumed. Pupae have the least variability. Data are not available to estimate the percentage of this stage present in wheat 25-26 days after the start of oviposition.

The dosage-mortality curves in PLATES IV and V were plotted using probits calculated by the IBM 650. Like the curves for the adults, they are plotted on logarithmic-probit graph paper. By plotting the calculated curves it is possible to estimate, as was done with the adults, the dosage required to give certain levels of mortality. LC_{90} values were estimated for the immature forms and are listed in Table 4. If susceptibilities of the immature

EXPLANATION OF PLATE IV

Dosage-mortality curves for rice weevil immature stages fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride in 12.5 percent moisture wheat.

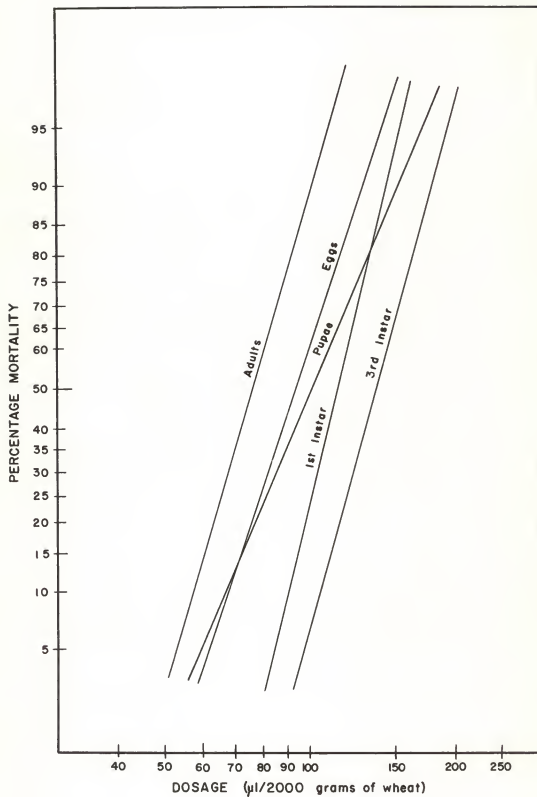
PLATE IV



EXPLANATION OF PLATE V

Dosage-mortality curves for rice weevil immature stages fumigated with an 80-20 formulation of carbon tetrachloride-methallyl chloride in 14.0 percent moisture wheat.

PLATE V



forms at LC_{90} are compared with those at LC_{50} it is noted that the order has changed. At both moistures the pupae are less susceptible in relation to the other stages tested. An indication of this was mentioned during the discussion of slopes. Orders of susceptibility at LC_{90} , least susceptible first, are as follows: 12.5 percent moisture -- third instar larvae < first instar larvae = pupae = eggs < adults; and 14.0 percent moisture -- third instar larvae < pupae < first instar larvae < eggs < adults. At both moistures, adults are most susceptible and third instar larvae least susceptible. The separation or spreading of order of susceptibility is evident for LC_{90} at 14.0 percent as well as at LC_{50} .

Although the tests were not designed to measure or evaluate the following observations, they should be mentioned. In several instances, at the lower dosages, there were greater numbers of insects emerging from the treated samples than from the controls. It appears as though the fumigant had some sort of stimulating effect on emergence. This could have been due to sampling error (variation) but the relative frequency of its occurrence leads to speculation. The tests using the immature insects also showed a distinct delay in peak emergence as dosages were increased. Both of these observations point to interesting aspects of immature insect fumigation studies and should be examined in greater detail.

SUMMARY

An accurate method for measurement of small quantities of fumigant was discovered in developing the fumigation procedure. The micro syringe used in measuring and applying the fumigant is a precision instrument, capable of measuring increments of 0.0001 ml. It has a total capacity of 0.25 ml. This

instrument allowed accurate measurement of small increments of the fumigant and rapid delivery to the fumigation chambers. Precise measurement makes it possible to conduct fumigation tests in small chambers and still obtain reproducible results. The amount of grain required for fumigation tests can be reduced by use of small size chambers thereby placing this type of testing on a practical laboratory scale.

The processed data indicated that an 80-20 formulation of carbon tetrachloride-methallyl chloride is slightly more toxic to adult rice weevils than carbon tetrachloride-carbon disulfide (80-20). It also appeared to be relatively toxic to immature forms; however, comparative data from other fumigants are not available. At LC_{90} the least susceptible immature form required 63 and 78 percent more fumigant than was required to kill 90 percent of the adults at 12.5 and 14.0 percent moisture respectively.

Considering the biological factors, it is possible that an 80-20 formulation of carbon tetrachloride-methallyl chloride could compete successfully with the "standard", an 80-20 formulation of carbon tetrachloride-carbon disulfide, as a grain fumigant. Economic factors such as cost of manufacturing, cost of products, and cost of selling have not been considered.

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EFFECTIVENESS OF AN 80-20 FORMULATION OF CARBON
TETRACHLORIDE-METHALLYL CHLORIDE AS A FUMIGANT
FOR RICE WEEVIL, SITOPHILUS ORYZAE (L.), IN WHEAT

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Fumigation tests were made to determine the effectiveness of an 80-20 (by volume) formulation of carbon tetrachloride-methallyl chloride as a fumigant for various life history stages of the rice weevil, Sitophilus oryzae (L.), in wheat. Rice weevil adults were fumigated in wheat of 11.0, 12.5, and 14.0 percent moisture for 48 hours at 80 degrees F. Eggs, first and third instar larvae, and pupae were fumigated in 12.5 and 14.0 percent moisture wheat.

A micro technique for fumigant application was discovered in developing the fumigation procedure. A micro syringe, capable of measuring units of 0.0001 ml., provided a means whereby small increments of fumigant could be measured accurately and delivered rapidly to fumigation chambers.

Mortality counts of adult rice weevils were made 0, 5, and 10 days after the fumigation exposure. Data from the 10-day counts were statistically analyzed with an IBM 650 Magnetic Drum Data Processing Machine by the method of maximum likelihood. Information from the processed data showed the 80-20 formulation of carbon tetrachloride-methallyl chloride to be more toxic than an 80-20 formulation of carbon tetrachloride-carbon disulfide to adult rice weevils. There was a direct relationship between toxicity to insects and moisture of the wheat. As moisture was increased, the amount of fumigant to kill a given percentage of the insects increased. The data also showed that most of the adult insects killed by the fumigant died within 5 days after the end of the fumigation. There was no apparent "knock-down" with subsequent recovery as is experienced with some fumigants.

Immature rice weevil data were processed in the same manner as the adult data. Order of susceptibility, based on LC_{50} , for all stages tested was as follows (least susceptible first): 12.5 percent moisture -- third instar

larvae < first instar larvae = eggs < pupae = adults; 14.0 percent moisture -- third instar larvae < first instar larvae < pupae < eggs < adults. For LC_{90} the order of susceptibility was: 12.5 percent moisture -- third instar larvae < first instar larvae = pupae = eggs < adults; 14.0 percent moisture -- third instar larvae < pupae < first instar larvae < eggs < adults. At LC_{90} , to kill the least susceptible stage required 63 and 78 percent more fumigant than to kill the adults in wheat of 12.5 and 14.0 percent moisture respectively.